High School Classroom Activities

Restriction Enzyme Mapping Lab

Introduction

In this lab, we will be observing how Restriction Enzymes, which are found in bacteria, cleave the double helix of DNA at specific places. These enzymes (proteins) bind to DNA at specific nucleotide sequences (recognition sequences) and break the sugar-phosphate backbone in each strand of the double helix. This results in a DNA molecule being cut into discrete pieces (fragments) of DNA of different sizes. The number of DNA fragments and the size of each fragment is dependent on the location and number of recognition sequences in that particular DNA molecule.

We will be using a double stranded circular DNA molecule, called a plasmid. The plasmid we are using is called **pFluoroGreen (or pFG)**, which is 6361 nucleotide base pairs in size. We will attempt to cut it into pieces using 2 different Restriction Enzymes: one called **Bgl I** from the bacteria *Bacillus globigii*, and another called **Eco RV** from Escherichia coli. By incubating the DNA with the restriction enzymes under specific conditions, the plasmid DNA will be cut into pieces. During the next lab we will separate the DNA fragments by **gel electrophoresis,** so we can determine the number fragments and the size of each.

Procedures

Each table will work together to set up 1 set of restriction enzyme digests. Each table has an ice bucket containing 5 tubes, one labeled **pFG** (the plasmid DNA), one labeled **Rxn Mix** (containing all the necessary components for the restriction enzyme to work), and 3 tubes with restriction enzymes; labeled **B**(Bgl I), **E** (EcoRV) and **B/E** (a mixture of BglI and Eco RV).

Part 1:

a) Label 3 microfuge tubes: **B**, **E**, and **B/E.** Put your groups initials tube as well.
b) Add **9 µl** of pFG (DNA) to each of the 3 tubes you labeled. Be sure to pipette carefully and accurately!
c) Add **10 µl** of Rxn Mix to each of the 3 tubes.
d) To the tube labeled B, add **1 µl** of the restriction enzyme BglI. Check with instructor that you have pipetted up only 1 µl! Mix contents and spin tube in centrifuge to get all liquid to bottom of tube.
e) To the tube labeled E, add **1 µl** of the restriction enzyme Eco RV. Mix contents and spin tube in centrifuge to get all liquid to bottom of tube.

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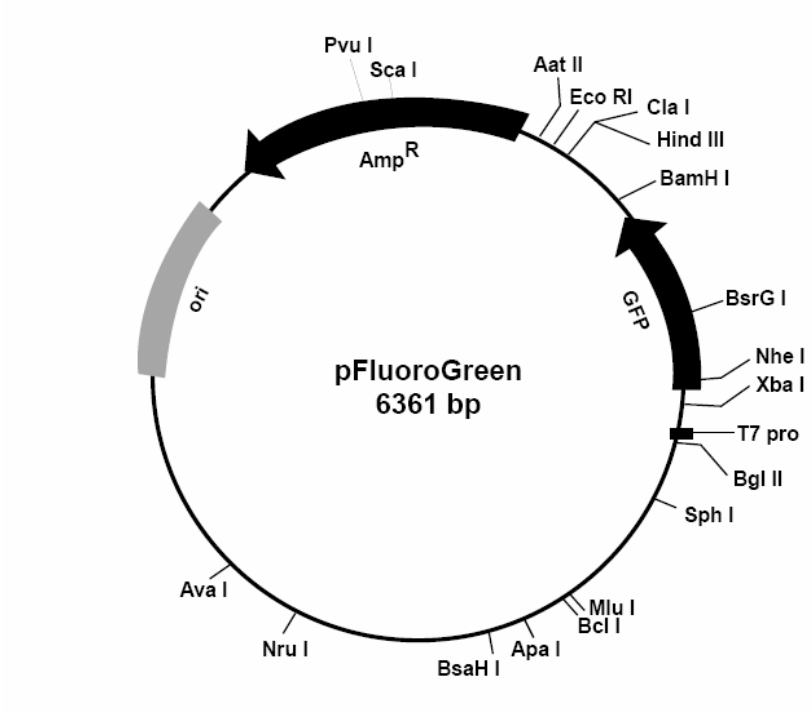
f) To the tube labeled B/E, add **1 µl** of the restriction enzyme mixture, B/E. Mix contents and spin tube in centrifuge to get all liquid to bottom of tube.

g) Incubate your 3 tubes in the 37°C waterbath using the styrofoam float for 40 minutes. (Your instructor will remove your tubes and freeze them until next lab.)

Plasmid: pFlouroGreen (6361 bp)

Restriction Enzymes:

- Eco RV (GAT'ATC) cuts at 205, 2337
- BglI (GCCN NNN'NGGC) cuts at 2951, 3185, 5504



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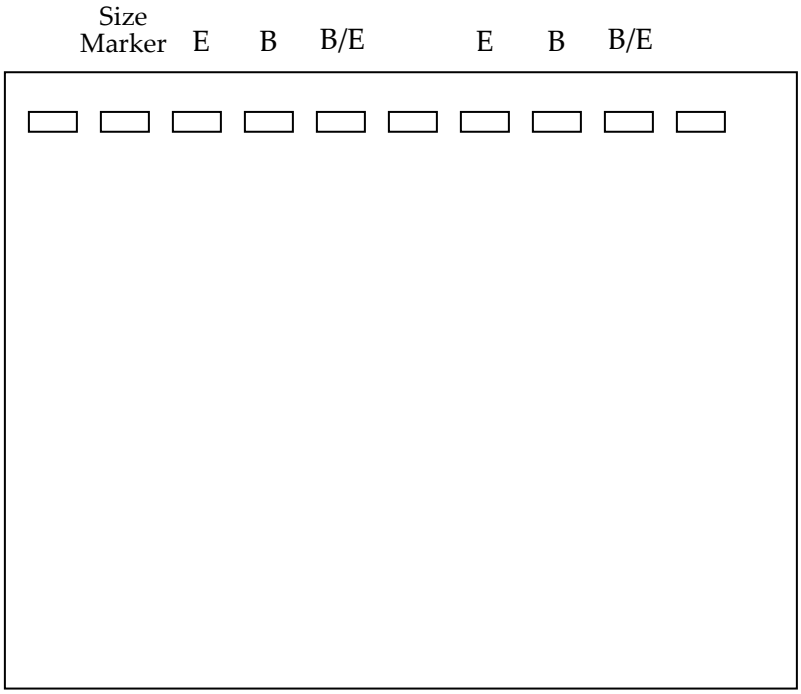
Part 2:

a) Obtain your 3 tubes from Tuesday's lab. Briefly centrifuge tubes to bring the contents to the bottom of the tube.
b) Add 5 µl of Loading Dye to each tube, mix, and centrifuge briefly.
c) It's time to load the gel! At least 2 groups will be loading their samples on the same gel. Each gel should have 1 lane loaded with the DNA size markers (load 6 µl of the size marker). Load the entire contents of your tubes (25 µl) in 3 consecutive wells, as shown on the diagram below.
d) Attach lid to electrophoresis chamber, and turn on power supply. Electrophorese for 40 minutes.
e) Stop electrophoresis, and observe the gel on the U.V. light box and photograph.



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Worksheet: Analysis of a Restriction Enzyme Digest of a Plasmid

Name: Period:

Introduction

In our experiment, we digested the plasmid pFG (pFluoroGreen) with two different restriction enzymes: Bgl I and EcoR V. Today we are using an agarose gel to separate the Digested DNA fragments by size.

Procedures

Your task is to predict what we will see on the gel – how many DNA fragments will there be, and how big will they be?

Part 1: How many DNA fragments?

Using the restriction map below, answer the following:

1. How many times does the restriction enzyme EcoR V cut plasmid pFG? (1 pt)

2. How many DNA fragments will there be after digesting with EcoR V? (hint – if you cut a circle once, you still have only one piece) (1 pt)

3. How many DNA fragments will there be after digesting with Bgl I? (2 pts)

Part 2: How big will they be?

The restriction map below shows the location in the plasmid where each enzyme cut will occur. For example, one EcoR V cut will happen at DNA base pair 205 – meaning that it happen at 205 nucleotide pairs away from the origin (which is always labeled at "0"). To figure out how long a DNA fragment will, be simply subtract the location of one cut from the location of

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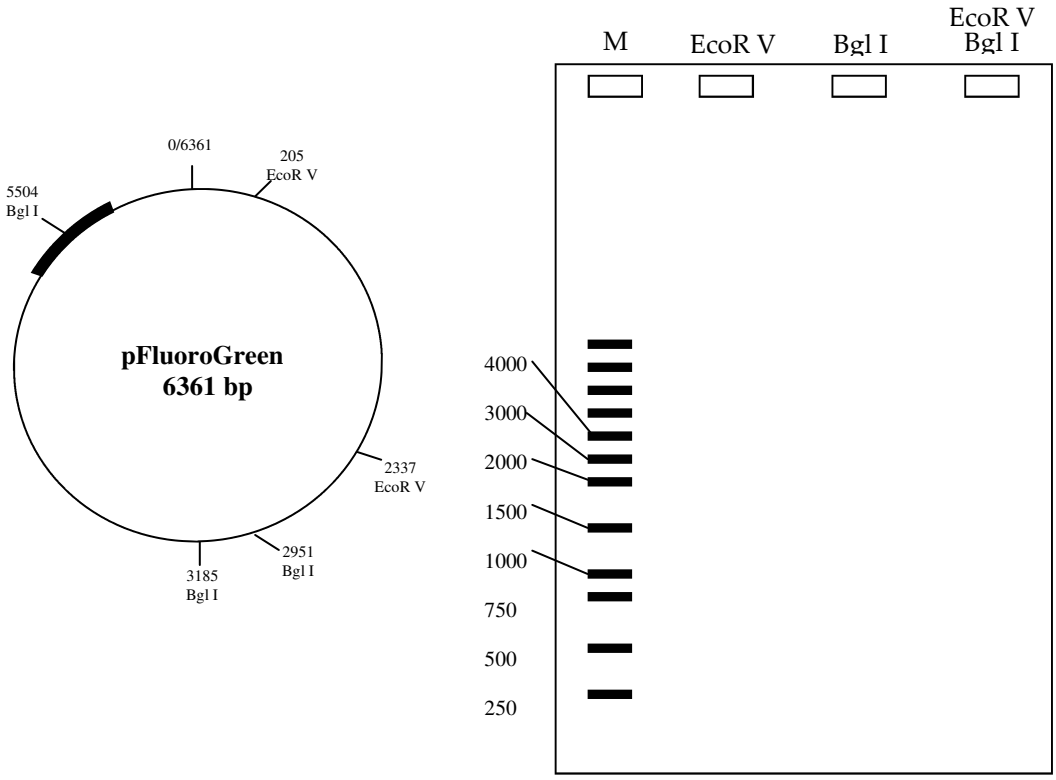
the other. Example: the smaller DNA fragment generated by the EcoR V digest will be 2337 – 205 = 2132 base pairs long. To calculate how large the other fragment will be, simply subtract the length of the first fragment from the total length of the plasmid. Example: the other EcoR V fragment will be 6361 – 2312 = 4049 base pairs long.

4. How long will the DNA fragments made by digesting with Bgl I be? (3 pts)

5. The sample agarose gel shows where the molecular weight standards will end up after the gel is run. Draw into each lane on the gel the DNA fragments that should appear. (1 pt for each lane)



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